



Origin, fate and ecotoxicity of manganese from legacy metallurgical wastes



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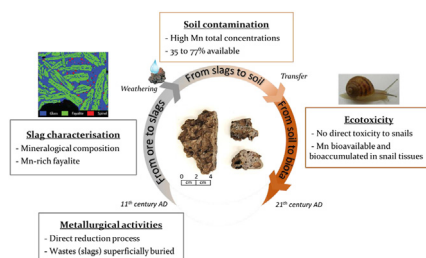
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HIGHLIGHTS

- Slags from the 11th century contain high amounts of Mn mostly found in fayalite.
- Slag weathering over centuries has led to Mn release and soil contamination.
- Ecotoxicity tests using snails revealed the bioavailability of Mn in soils.
- Sites of past metallurgical activities remain a cause of environmental concern.

GRAPHICAL ABSTRACT



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ABSTRACT

Over the course of history, mining and metallurgical activities have influenced the socioeconomic development of human populations. However, these past and current activities can also lead to substantial environmental contamination by various metals. Here, we used an interdisciplinary approach (incorporating archaeology, mineralogy, environmental chemistry and ecotoxicology) to investigate the origin, fate and potential ecotoxicity of anomalous manganese (Mn) concentrations detected in the ancient mining district of Berthelange (medieval period, eastern France). Mineralogical investigations of slag samples showed that smelting temperature conditions in medieval bloomeries led to the production of slags mainly composed of Fe- and Mn-rich olivine, *i.e.*, fayalites. Further mineralogical analyses of bulk soil and clay fractions allowed us to identify the presence of serpentine. This evidence of olivine weathering can account for the release of Mn from slags into the soil. In addition, chemical analyses of total and available (exchangeable and reducible) Mn concentrations in soil samples clearly showed the contribution of slags deposited 1000 years ago to soil contamination. A complementary ecotoxicity bioassay performed on soils from a slag heap using the land snail *Cantareus aspersus* confirmed that a significant fraction of the Mn detected in soils remains available for partitioning with the soil solution and transfer to soil organisms. Although no growth inhibition of snails was observed after 28 days of exposure, the animals accumulated quite elevated Mn concentrations in their tissues. Our study emphasizes the environmental availability and bioavailability of Mn from ancient metallurgical wastes to soil-dwelling invertebrates, *i.e.*, snails, even one millennium after their deposition. Hence, as for more

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recent industrial sites, past mining ecosystems must be a cause of concern for the scientific community and public authorities.

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1. Introduction

Throughout human history, and especially since the Bronze Age, socioeconomic development has been strongly influenced by the exploitation of mineral resources, such as copper (Cu), iron (Fe), lead (Pb) or silver (Ag) (Nriagu, 1996; Tylecote, 1987). However, mineral-related activities also constitute the most ancient and significant source of waste production (Macklin et al., 1997; Pyatt et al., 2005). These types of wastes, originating from mining (ore dumps, ore-washing sediments) or metallurgical (smelter dusts, slags) operations, are currently considered archaeological clues. Until quite recently, slags were mostly considered chemically inert because of the strong association of metals with glass and mineral phases, limiting their lability (Wilson et al., 1994). However, a growing body of evidence shows that slags are affected by weathering and may be able to release metals into aquatic and terrestrial compartments (Ettler, 2002, 2016; Gee et al., 1997; Lottermoser, 2002; Parsons et al., 2001; Piatak et al., 2004; Seigneur et al., 2006).

In addition to the modification of the geochemical cycles of metals, this contamination may be responsible for the deterioration of water and soil quality and the impairment of biodiversity (Ettler et al., 2009; Lottermoser and Cairns, 2005). For instance, Monna et al. (2011) showed that wild brown trout living in past mining areas located in the Cevennes (France) were contaminated by Pb originating from historical activities (15th and 20th centuries). More recently, Mariet et al. (2016 and 2020) highlighted that metals originating from mining operations (Pb and Ag) during the end of the Middle Ages in the Vosges Mountains (France) remain bioavailable to soil invertebrates. However, the older the sites are, the scarcer the data are. In the Franche-Comté region (eastern France), Fe mining and metallurgical activities began in the Early Middle Ages (5th century), with particular intensity in the district of Berthelange (Forlin and Laurent, 2014; Laurent, 2016). Until the 15th century, a direct ore-reducing process was conducted within clay-made blast furnaces. In these bloomeries, temperatures exceeded 1000 °C, allowing, with the addition of charcoal, the reduction of Fe oxides (Fe₂O₃). At the end of the smelting process, a heterogeneous block of Fe (sponge) was kept for further purification, and metal-rich residues, *i.e.*, slags, were dumped on site as heaps. Currently, more than 1000 years later, the environmental effects of potential metal releases have, to our knowledge, not been evaluated.

Therefore, this study aims to assess the effect of very ancient slag deposits on soil quality. Because a single chemical analysis of total metal concentrations in soils may be insufficient for ecological risk assessment purposes (Peijnenburg, 2020), we combined physical, chemical and biological tools in a holistic approach. We operationally investigated the metal distribution in and transfer between various matrices (slags, soil and invertebrates) to reveal the origin, fate and effects of legacy contamination.

2. Materials and methods

2.1. Study area and sampling design

This study was performed in the Bourgogne Franche-Comté region of eastern France, where Fe mining and metallurgical

exploitation were important activities during the Middle Ages (Jacob and Mangin, 1990). Over this period, Fe mining and metallurgical activities were particularly intense in the district of Berthelange (140 km², 20 km west from Besançon). The study area is composed of Jurassic limestones and marls (from Toarcian to Bajocian) partly covered by clay and sandy alluvial Pliocene deposits (Dreyfuss and Kuntz, 1967). Here, more than 120 direct Fe ore-reducing process sites were identified (Forlin and Laurent, 2014) because of the presence of slag repositories (from 10 to 20 m in diameter) (Fig. 1A). In this study, eleven slag repositories, located in forested regions and well preserved, were investigated for the detection of potential anomalous metal concentrations in soils (Fig. 1A, Table 1). Among them, the Antorpe 9 heap (Fig. 1B), containing slags produced during the 11th century (Forlin and Laurent, 2014), was selected for further investigations. More precisely, slags and soils were sampled using a transect method following four directions (NE, SE, SW and NW) from the centre of the slag heap (sampling point, SP, 4) according to Fig. 1C. The reference site SP10 was located 100 m west of the heap. This remote station was selected to represent the pedogeological background of the area studied, *i.e.*, the same geological substrate and same vegetation cover, but without any smelting activities or slag deposit. At each sampling point, three soil cores (0–100 cm) were taken (after removing surface vegetation and humus) using a soil auger.

2.2. Soil analyses

2.2.1. Physico-chemical characterisation

The soil samples were primarily sieved (2 mm mesh; slag fragments were sorted for further analyses), dried at 40 °C and homogenized. The soil pH was measured using a pH metre (WTW, pH/ION 3310) after extraction with demineralized water (1:5, v:v; ISO 10390, 2005), and the organic matter (OM) content was determined via the loss of ignition (LOI) method according to NF EN 15935 (AFNOR, 2013). Total metal concentrations were determined after hot mineralization in *aqua regia* (1:2.5 HNO₃: HCl, v:v) using a block digestion system (DigiPREP) and filtration (1 µm) by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Thermo Scientific, iCAP 6000 Series). The reliability of the analyses was assessed with certified reference material (calcareous loam soil: CRM n°141R from BCR). The recovery rate was on average 107 ± 8% for all the metals analysed.

To identify potential decay products of slag minerals, mineralogical analyses of soil samples (bulk and clay fraction) from different depths (0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm and 80–100 cm) were obtained using X-ray diffraction (XRD) characterisation and a D8 Advance Bruker diffractometer equipped with a LinxEye detector (CuK α radiation at 1.54184 Å, 40 kV/40 mA) hosted at the Utinam Institute (University Bourgogne Franche-Comté, France). Powdered bulk rock samples were treated between 2 θ values of 3° and 65° (divergent slit) with a step scan of 0.019° (2 θ) and a scan rate of 1 s/step. The EVA software package was used for data processing and phase identification. The extraction of clay fractions required prior OM destruction by successive addition of 1 cm³ hydrogen peroxide (33% H₂O₂, VWR Chemicals) to hydrated soil samples previously blended with water (1:5; w:v). To increase

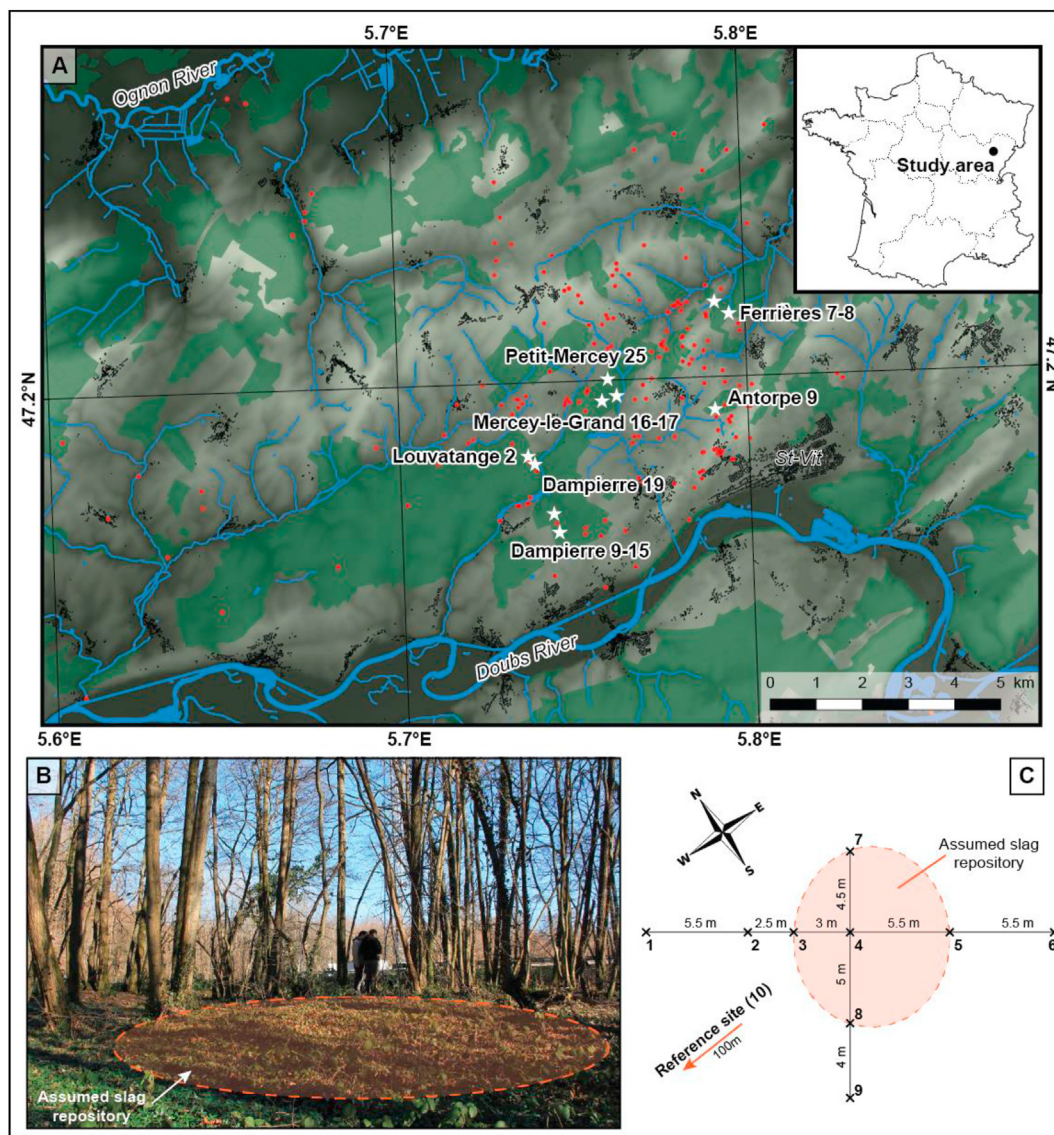


Fig. 1. A: map of the Berthelange ancient metallurgical district. Green areas are forests, red dots correspond to archaeologically referenced slag deposits (from Forlin and Laurent, 2014) and white stars correspond to the investigated slag heaps. B: photograph of the Antorpe 9 slag heap. C: sampling map of the Antorpe 9 heap. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the reaction rate (Holtzapffel, 1985), this mixture was heated at 60 °C on a digital hotplate (Stuart SD500). The samples were then subjected to repeated washing ($n = 4$) using ultrapure water (UP) and centrifugation (10 min, 8000 rpm). Afterward, clay suspensions were shaken in an end-over-end shaker for 10 min at 10 rpm. Finally, two clay fractions ($<16 \mu\text{m}$ and $<2 \mu\text{m}$) were obtained by the sedimentation process according to Stoke's law, which links the settling rate to the particle size, and mounted on glass slides for XRD analyses. Before successive XRD analyses, the oriented preparations were sequentially air dried, glycolated and heated for 4 h at 490 °C for identification of the interlayer of clays, *i.e.*, the (001) basal reflection positions after each treatment. The XRD patterns were obtained between 2° and 32° (2θ) with a step scan of 0.009° (2θ) and a scan rate of 1.5 s/step.

2.2.2. Environmental availability

The environmental availability was estimated using a chemical extraction method chosen to quantify the potential of metals for leaching or uptake by plants (Abedin et al., 2012). The extraction

solution consisted of 1 mol.L^{-1} ammonium acetate (NH_4OAc , purity $>99\%$) added to a solution containing 2 g.L^{-1} hydroxylamine hydrochloride salt (NH_2OH , HCl, purity $>99\%$) and buffered at $\text{pH} = 7 \pm 0.05$ using ammonium hydroxide (NH_4OH , purity $>99\%$). Soil suspensions were obtained after shaking 37.5 mL of extraction solution and 1.5 g of dried soil in an end-over-end shaker for 2 h at 10 rpm. Suspensions were then centrifuged at 3000 rpm for 10 min and filtered through a $0.45 \mu\text{m}$ cellulose acetate disk filter to obtain the extracts (AFNOR, 2002). To ensure complete extraction, the soil residues were subjected to four successive extractions of 2 h each. Finally, metal concentrations were determined in the total extract solutions by ICP-AES.

2.3. Slag analyses

2.3.1. Total metal concentrations

Total metal concentrations in slags (bulk) were determined according to the international standard ISO 14869-1 (2001). Briefly, 0.250 g milled slag samples ($n = 3$) were digested in PTFE dishes on

Table 1

Metal concentrations ($\mu\text{g.g}^{-1}$) in soils from slag heaps and reference sites of the Berthelange district and in slags (bulk) from the Antorpe 9 repository^a; data from Crommentuijn et al. (2000)^b; ecological soil screening level (Eco-SSL) from the US EPA (2007). < DL: below detection limit.

	As	Ca	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Ref 1	13.3	268.6	<DL	8.3	11.7	16,811.6	1402.7	483.5	12.4	41.4	33.2
Ref 2	26.2	403.7	<DL	16.8	20.3	32,814.5	1390.4	789.2	21.3	48.9	62.9
Ref 3	19.2	335.4	<DL	16.4	15.4	25,574.8	1849.5	1146.5	18.5	28.2	47.6
Mercey-le-Grand 16	21.9	995.7	<DL	14.4	19.7	36,936.9	1663.0	1172.4	20.6	31.0	69.1
Ferrières 7	23.3	775.9	<DL	22.4	19.0	35,746.0	1850.6	1208.2	25.9	26.6	195.7
Dampierre 19	23.1	5461.2	0.6	15.0	21.3	27,745.6	1622.5	1422.5	24.4	29.8	61.2
Ferrières 8	35.4	797.4	0.3	22.5	21.0	40,902.3	1704.1	2088.4	32.3	31.6	135.7
Mercey-le-Grand 17	25.0	573.8	<DL	18.3	25.0	57,745.1	1391.5	2104.3	23.4	30.7	91.6
Petit-Mercey 25	57.5	3807.3	<DL	24.0	29.5	60,975.6	1600.6	2236.7	31.0	40.3	104.1
Louvatange 2	20.0	923.2	0.8	18.1	25.3	38,756.5	1294.2	2602.0	24.6	28.6	78.0
Dampierre 15	9.5	1075.6	<DL	14.3	16.9	32,110.0	1051.2	3165.8	16.9	31.2	70.2
Antorpe 22	30.1	3369.2	0.2	20.4	38.8	82,160.3	2027.5	3207.0	36.4	38.3	121.0
Dampierre 9	16.8	2152.3	<DL	16.4	26.3	52,950.5	1383.0	4709.2	20.3	55.2	87.2
Antorpe 9	51.8	6618.9	2.2	37.9	65.4	137,095.6	1553.7	8575.6	64.8	41.7	244.8
Maximum Permissible Concentration ^a	34.0		1.6	20.0	40.0			450 ^b	30.0	50.0	50.0
Antorpe 9 Slag #1	<DL	12,665.0	3.3	6.9	86.3	149,243.0	3296.6	17,590.5	27.3	11.5	27.2
Antorpe 9 Slag #2	<DL	14,899.2	3.3	10.8	87.4	154,248.9	3759.9	22,070.9	31.2	13.3	36.0
Antorpe 9 Slag #3	<DL	18,485.5	2.9	6.2	76.4	137,505.3	3436.2	11,135.5	25.0	9.0	28.8

a hot plate (150 °C) after the addition of 5 mL of hydrofluoric acid (47% HF) and 1.5 mL of perchloric acid (65% HClO₄). The mixture was allowed to evaporate to near complete dryness, and the residue was dissolved in diluted nitric acid (HNO₃) before analysis by ICP-AES. The reliability of the analyses was assessed with certified reference material (calcareous loam soil: CRM n° 141R from BCR). The recovery rate averaged 104 ± 6% for all the metals analysed.

2.3.2. Mineralogical characterisation

Slags were first ground, and the whole-rock powders were used for XRD analysis. XRD patterns were obtained using the same method as for soil mineralogical characterisation and allowed assessment of potential slag decay within soil through mineralogical analysis (comparison of similar mineralogical phases within soil and slag samples).

Then, hand samples of slags were cut to prepare a 30 µm-thick polished thin section. Optical investigations using scanning electron microscopy (SEM) in back-scattered electron (BSE) mode were conducted at the Femto-ST Institute (University Bourgogne Franche-Comté, France). Observations were complemented by energy-dispersive spectrometry (EDS) mapping obtained at the University of Lausanne (Switzerland) using a Tescan Mira LMU SEM equipped with a field emission gun (FEG). The composition of slag-forming minerals and glass were obtained by electron probe microanalysis (EPMA) at the University of Lausanne using a JEOL 8200 Superprobe equipped with five wavelength dispersion spectrometers (WDS). Carbon-coated polished thin sections were analysed with a 15 kV beam and a current of 15 nA. The resolution was ca. 1 µm. Oxides and silicates were used as standards. In addition, X-ray intensity mapping was carried out to reveal the specific textures caused by the chemical features of minerals.

2.4. Ecotoxicity test: snail growth inhibition and bioaccumulation

2.4.1. Animals

Land snails (*Cantareus aspersus*, Müller 1774, syn. *Helix aspersa*) were reared under controlled conditions according to Gomot-de Vaufleury (2000). For the bioassay, juvenile snails (n = 105) were reared for 4 weeks and weighed 1.05 ± 0.15 g prior to exposure.

2.4.2. Exposure modalities

Following the standard ISO 15952 (2018), the juvenile snails

were exposed for 28 days to three surface soils (0–20 cm) originating from increasing distance to the centre of the smelting waste repository, i.e., presenting a gradient in metal contamination (SP10, 8 and 4), and to a control treatment (C) in which snails were sustained over dampened absorbent paper. For each treatment, five transparent polystyrene containers of 3200 cm³ (24 × 21 × 8 cm; ref. E1DBBAC001, Charles River IFFA-CREDO, 69 L'Arbresle) were used. Each container was filled with a 1.5 cm layer (200 g dry weight, DW) of soil or an absorbent paper layer, and five snails were introduced. The snails were fed ad libitum with uncontaminated Helixal® snail food placed in a Petri dish left on the bottom of the container. The day/night cycle photoperiod was 18/6 h, the temperature was 20 ± 1 °C, and the air relative humidity was 90 ± 5%. Three times a week, the containers were cleaned, the faeces were removed, and the food was renewed.

2.4.3. Measured endpoints

Each week, individual snail fresh mass (whole-body) and shell diameter were monitored as biological endpoints for the assessment of sublethal effects.

At the end of the exposure, one snail was randomly sampled in each replicate container for each exposure modality and subjected to a fasting period of 48 h to empty its gut (the faeces were removed after 24 h to prevent their ingestion), then sacrificed by freezing at –80 °C. After thawing, the whole soft body was removed from the shell and separated into two parts: the viscera (i.e., the visceral complex containing the posterior gut, digestive gland, kidney, mantle, and part of the reproductive tract) and the foot (containing the foot *sensu stricto*, anterior gut, and remainder of the genital tract). The samples were then freeze-dried and digested in HNO₃ (purity 99.9%) before analyses for metal content by ICP-AES. The certified reference material was TORT-2 (lobster hepatopancreas from NRCC-CNRL, Canada), and recovery rates ranged between 90% and 118% of the certified values for all the metals.

2.5. Statistics

All data are presented as the mean ± standard deviation (sd). First, correlation analyses were performed using the Spearman rank correlation test between the slag abundance observed at each sampling point and physico-chemical parameters, such as the pH, OM content and total and extractable metal concentrations.

Significant differences among soil parameters, metal concentrations, snail growth and metal bioaccumulation were then analysed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test for pairwise comparisons. For the case in which the normality of distribution and homogeneity of variances were not verified (using the Shapiro–Wilk and Bartlett tests, respectively), the Kruskal–Wallis test followed by the Kruskal–Wallis post hoc test (pgrimm package) were used. For all statistical analyses, a *p*-value of 5% was used as a significance threshold.

All statistical analyses were performed with the free statistical software R (ver 3.4.0) (R Core Team, 2020).

3. Results

3.1. Soil contamination in the Berthelange district

Metal concentrations in soils formed above the slag heaps and in reference soils, *i.e.*, soils that had never been subject to metallurgical activities, are presented in Table 1. In the reference sites, metal concentrations are relatively low in comparison with values measured on slag repositories. Indeed, among the metals that have been analysed, some exceed the maximum permissible concentrations in soils. For instance, low anomalous concentrations are observed for arsenic (As) and zinc (Zn) in Petit-Mercey 25, Antorpe 9 or Ferrières 8. The most marked contamination concerns major elements, such as Fe and Mn, with total concentrations in soils of the latter element reaching more than 8500 $\mu\text{g g}^{-1}$ at the Antorpe 9 site. Therefore, further results and discussion will focus particularly on this site and this element.

3.2. Characterisation of slags

At the macroscopic scale, slags from the Antorpe 9 heap are generally homogeneous in shape, density and porosity. The morphology directly reflects the smelting technology in bloomeries. Slags are generally dark-coloured, ranging from blueish to brownish. Flow texture is frequently observed and is characteristic of slags formed in a molten state. Vesicles, ranging from micrometres to centimetres, are commonly met.

Metal concentrations in slags of Antorpe 9 (Table 1) reach an average of approximately 15% Fe and 1.7%, 1.5% and 0.35% Mn, Ca and Mg, respectively. Other quantified trace metal elements present lower concentrations ranging between 0.0003% (for cadmium, Cd) and 0.008% (for Cu). At the mineralogical scale, XRD patterns allowed us to identify fayalite, an olivine group mineral, as the main component of slags (Fig. 2A). Petrographic investigations on polished thin sections showed that the phases present in Antorpe 9 slags include olivine, glass, spinel, leucite, wustite and metallic Fe (Fig. 3). Olivine group minerals commonly display a spinifex texture and elongated skeletal laths; the WDS analyses revealed a fayalite (Fe_2SiO_4) composition. The glass phase is generally interstitial and contains subhedral spinel crystals (Fig. 3B). Locally, wustite occurs as bleb aggregates and is partially replaced by secondary Fe phases. Similarly, metallic Fe fragments are corroded and transformed into oxides and hydroxides (Fig. 3D). As shown in Table 2, Mn is heterogeneously distributed between minerals and glass. Fayalite, which may contain up to 5% MnO, is the primary carrier of Mn, while glass and spinel display MnO contents of less than 1.8% (Table 2; Fig. 3C). Manganese is almost absent in metallic Fe and secondary Fe phases (Fig. 3F).

3.3. Influence of the slag heap on soil characteristics

The edges of the heap were delimited using the relative

abundance of slags in the surface layer (0–20 cm) of soils collected at each sampling point (Fig. 4). Regardless of the transect direction considered, the slag abundance decreases with the distance from the centre of the heap, dropping from approximately 70% at SP4 to less than 5% at the heap foot (SP3, 5, 7 and 8).

Significant positive correlations were identified between the slag abundance and some physicochemical parameters of soils (Fig. 4), such as the OM content ($\text{Rho} = 0.94$, $p < 0.001$) and pH ($\text{Rho} = 0.54$, $p < 0.001$). Indeed, from the edge of the slag heap to its centre, the OM content and pH values increase from 8 to 19% and 4.8 to 5.4, respectively.

Variations in the clay composition of soils are also observed by means of XRD analyses of soils (Fig. 2B) and clay extract (Fig. 2C–G). Mineralogical analyses of clay extract from soil samples allowed us to identify different clay minerals that are present in various proportions depending on the samples: illite, chlorite, kaolinite, serpentine (antigorite/chrysotile), and mixed-layer clay minerals. Kaolinite and serpentine exhibit very similar diffraction patterns, but the absence of tetrahedral substitution in antigorite and chrysotile induces rolling up of the layers (Holtzapffel, 1985; Moore and Reynolds, 1997). This characteristic allowed us to distinguish samples without serpentine (Fig. 2E) from samples containing serpentine (Fig. 2F and G).

The total Mn concentrations in the soils range from 1150 $\mu\text{g g}^{-1}$ at the reference site (SP10) to approximately 3500 $\mu\text{g g}^{-1}$ at the heap foot (SP3, 7 and 8) and up to more than 8000 $\mu\text{g g}^{-1}$ at the centre of the heap (SP4) (Fig. 4). Manganese extractable concentrations display a similar pattern to total Mn with increasing values from SP10 (880 $\mu\text{g g}^{-1}$) to SP4 (2800 $\mu\text{g g}^{-1}$), representing extraction yields of 77% and 35%, respectively. Surprisingly, at SP9, the total and extractable concentrations were particularly low, *i.e.*, 400 and 170 $\mu\text{g g}^{-1}$, respectively.

3.4. Ecotoxicological bioassay

No snail mortality was recorded over the 28-day exposure periods. Concerning sublethal effects, the growth of snails exposed to the four modalities did not show any significant difference either in terms of body mass (Fig. 5A) or shell diameter (data not shown). Indeed, the final mass of snails reached 6.9 ± 1.2 g for the control modality and 7.3 ± 1.4 g, 7.2 ± 1.6 g and 7.2 ± 1.1 g for snails exposed to soils located at 100 m, 5 m and the centre of the heap, respectively. Similarly, the final shell diameter increased 28.12 ± 3.0 mm for the control and 28.85 ± 2.38 mm, 28.92 ± 2.52 mm, and 29.29 ± 1.97 mm for snails exposed to the three previously cited soils.

Growth measurements were completed with Mn bioaccumulation data in snail foot and viscera after 28 days of exposure (Fig. 5B). As snails grew over the 28-day duration of the exposure, we used body burdens rather than tissue concentrations because the latter can be affected by rapid changes in the mass of the organism (Gimbert et al., 2006; Gimbert and de Vaufleury, 2009). Snails significantly accumulate Mn in their viscera following a dose-response pattern according to the Mn concentrations in soils. Viscera internal burdens reached 25.2 ± 8.3 μg in the control snails (*i.e.*, snail food exposure only) and 93.4 ± 1.1 μg , 93.5 ± 29.6 μg and 154.0 ± 34.4 μg in snails exposed to soils from SP10, SP8 and SP4, respectively. Accumulation in the foot is low, and body burdens do not vary regardless of the exposure modality (Fig. 5B).

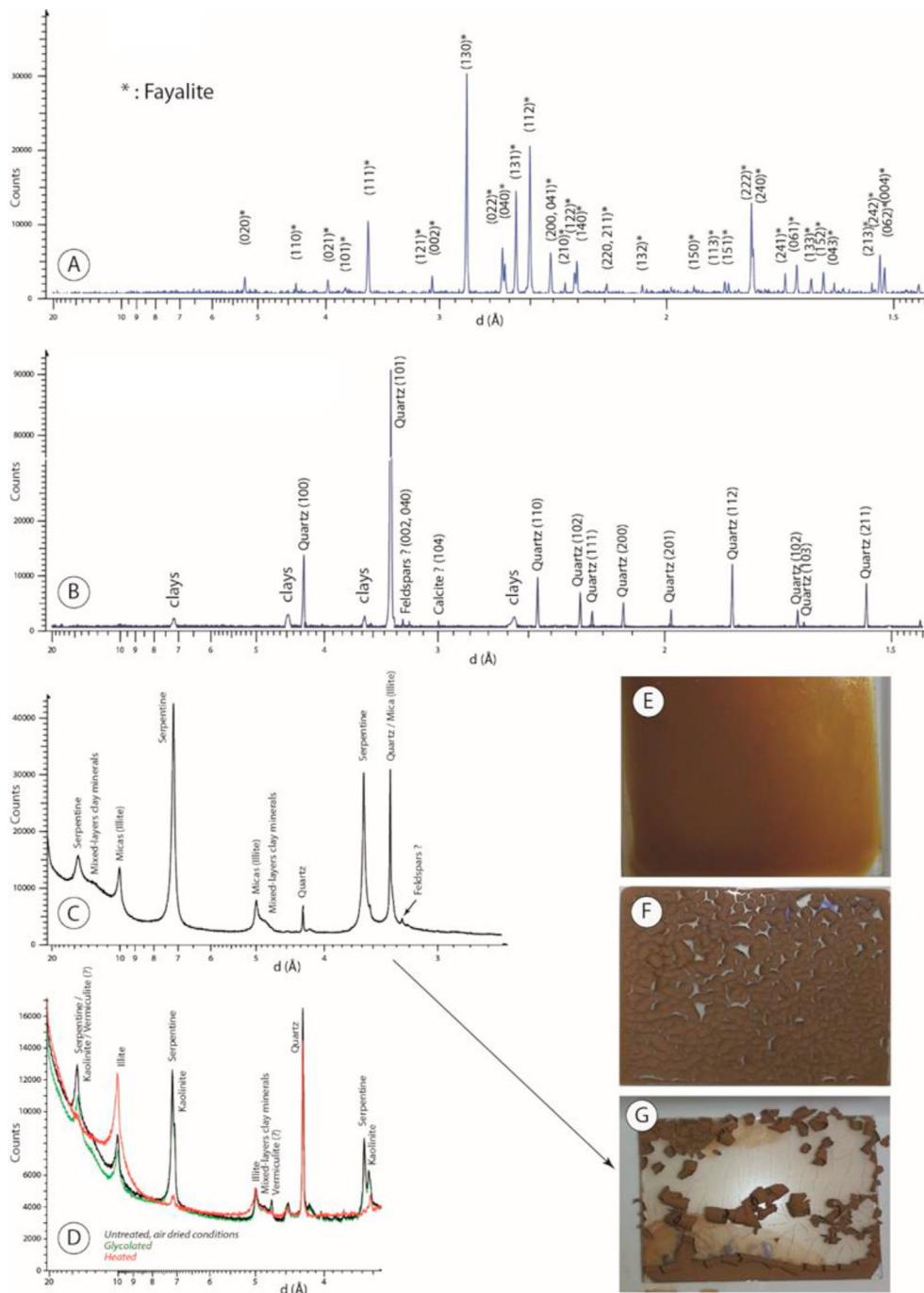


Fig. 2. Diffractograms. A: slag; B: bulk soil (SP8, 40–60 cm); C and D: clay fraction in soil (SP8, 40–60 cm and SP4 80–100 cm, respectively); E: photograph of glass slide with clay fraction without serpentine (SP10 0–20 cm); F and G: photograph of glass slide with clay fraction containing serpentine (SP4, 80–100 cm and SP8 40–60 cm, respectively).

4. Discussion

4.1. Source of Mn contamination in soils

Manganese concentrations measured in reference soils from the study area average approximately $800 \mu\text{g g}^{-1}$ and are in accordance with the Mn pedogeochemical background of soils (Pinsino et al., 2012; Grygo-Szymanko et al., 2016). Varying from 480 to $1150 \mu\text{g g}^{-1}$, these background concentrations nevertheless show a spatial heterogeneity that has to be related to the lithological nature of the soils (including within the same geological formation)

and to the local soil features (both abiotic and biotic). However, this natural variability is not of the same order of magnitude as what is observed in soils formed on ancient slag repositories. Indeed, Mn concentrations range from 1200 to more than $8000 \mu\text{g g}^{-1}$, testifying to the influence of smelting wastes on soil contamination. These values exceed the maximum permissible concentrations in soils; therefore, it is necessary to better understand the origin and fate of this element in past mining ecosystems.

In slags, three main mineralogical phases are identified: metallic Fe residues, glass and fayalites belonging to the olivine group. The presence of metallic Fe inclusions in slags attests to the generally

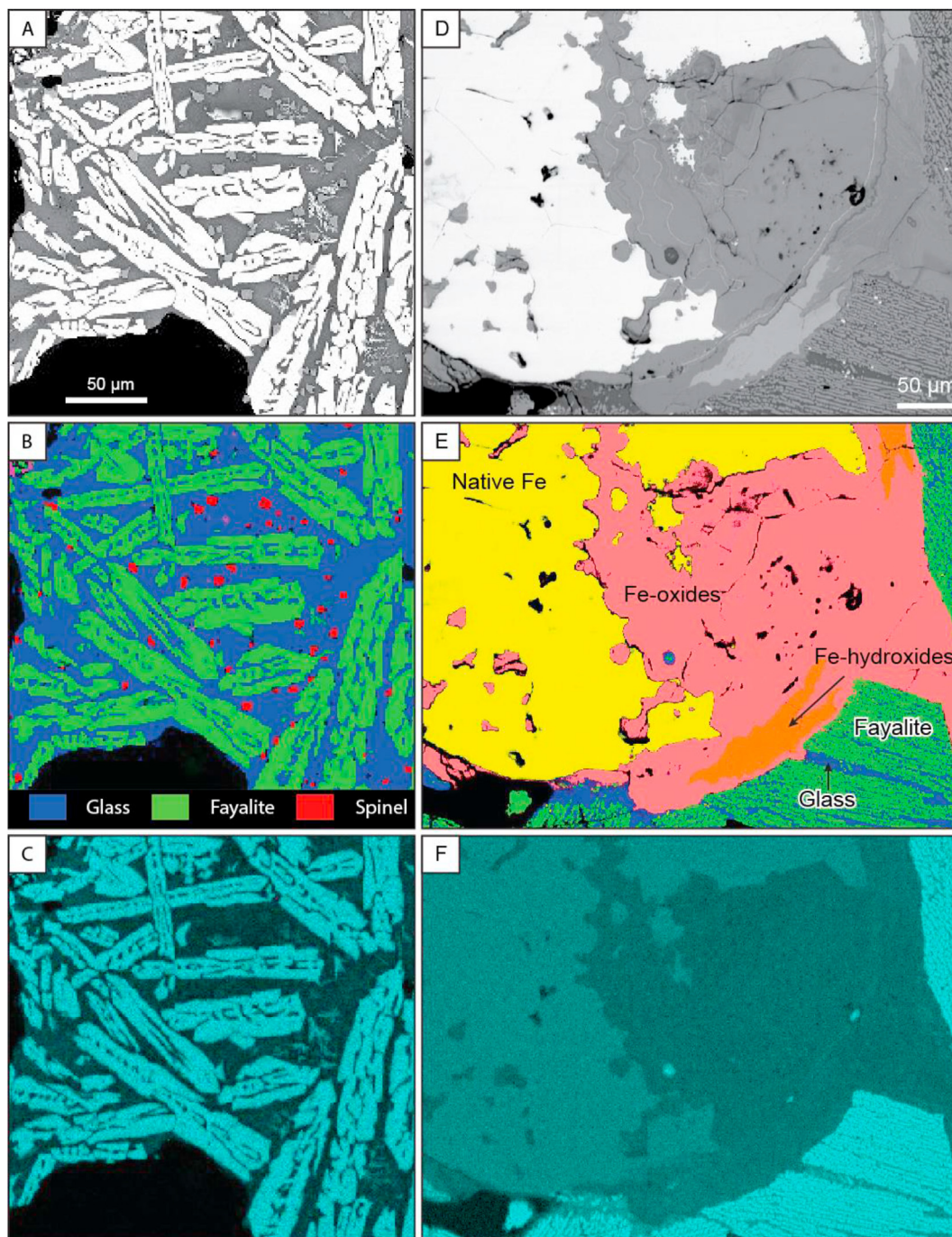


Fig. 3. Left (A to C): representative view of mineral-rich slag material from Antorpe 9. Right (D to E): detailed view of a spherical metallic Fe particle trapped within slag from Antorpe 9. A and D: BSE images; B and E: interpretative map of the mineral phases based on WDS and compositional maps, respectively; C and F: Mn X-ray composition map based on WDS and EDS, respectively. Brightness correlates with Mn content.

moderate extraction yields of Fe during the Early Middle Ages. Glass is ubiquitous in slags from Antorpe 9 and is primarily composed of Si, Ca and Al oxides, as reported in the literature (Piatak et al., 2015). The presence of fayalite and its dendritic shape are related to the temperature in the bloomeries (reaching more than 1000 °C) and the slow air-cooling conditions of molten slags at the end of the smelting process (Piatak et al., 2004; Tossavainen et al., 2007). The manganese concentration is 1.7% in slag bulk samples and is primarily located in fayalites, where it reaches 4.5 wt% MnO. In their review, Piatak et al. (2015) provided average concentrations of Mn

in steel and pre-1900 and modern Fe slags between 1 and 4 wt% MnO.

The weathering of the slag primary phases after abiotic (air or water) or biotic (microorganisms) interactions leads to the production of secondary products that can be used as indicators of the reactivity and alteration of slags (Piatak et al., 2015). In Antorpe 9 soils, three clues of slag alteration have been identified. First, the alkalization of soil (+0.6 pH unit between the reference soil and the centre of the heap) may originate from the hydrolysis of CaO and other Ca–Mg oxide and silicate phases of slags and the subsequent

Table 2
Chemical composition (mean ± standard deviation, SD) of slag-forming minerals and glass obtained by EPMA (% in weight).

	SiO2	K2O	Na2O	P2O5	MnO	Al2O3	CaO	MgO	TiO2	FeO	Cr2O3	NiO	Total
Glass (n = 4)													
mean	40.16	3.12	0.25	1.55	1.69	20.23	9.07	0.02	1.22	22.35	0.01	0.02	99.68
SD	2.68	0.73	0.05	0.20	0.08	2.85	1.46	0.02	0.10	2.30	0.01	0.03	0.21
Fayalite (n = 7)													
mean	29.24	0.07	0.01	0.52	4.51	0.59	0.51	1.00	0.13	63.72	0.02	0.01	100.32
SD	0.43	0.11	0.02	0.38	0.88	0.76	0.11	0.60	0.07	1.25	0.02	0.01	0.60
Leucite (n = 3)													
mean	55.10	18.65	0.12	0.15	0.14	25.52	0.11	0.02	0.06	2.15	0.01	0.00	102.02
SD	1.85	1.10	0.03	0.18	0.14	0.37	0.12	0.01	0.03	2.31	0.01	0.00	0.50
Spinel (n = 4)													
mean	3.01	0.13	0.02	0.02	1.56	49.23	0.08	0.25	2.41	44.60	1.02	0.00	102.32
SD	1.94	0.15	0.03	0.02	0.07	2.57	0.02	0.11	0.85	3.23	0.59	0.01	1.07
Wustite (n = 2)													
mean	3.47	0.04	0.02	0.73	0.68	7.65	0.55	0.08	0.59	87.11	0.02	0.01	100.95
SD	2.19	0.01	0.02	0.58	0.06	7.93	0.39	0.02	0.18	5.13	0.01	0.01	1.83
Metallic Fe (n = 2)													
mean	0.87	0.03	0.00	1.46	0.13	1.49	0.08	0.03	0.05	125.06	0.08	0.05	129.30
SD	0.39	0.03	0.00	1.91	0.15	2.10	0.10	0.02	0.06	1.89	0.12	0.07	1.54
Fe oxides (n = 4)													
mean	2.21	0.01	0.04	0.09	0.05	0.01	0.02	0.02	0.01	87.65	0.01	0.02	90.12
SD	1.11	0.01	0.05	0.18	0.02	0.01	0.01	0.02	0.01	1.66	0.01	0.01	0.79
Fe hydroxides (n = 4)													
mean	6.53	0.07	0.09	0.64	0.29	0.55	0.11	0.03	0.00	73.32	0.01	0.03	81.65
SD	4.08	0.04	0.10	0.77	0.23	1.06	0.04	0.03	0.01	2.86	0.01	0.03	1.45

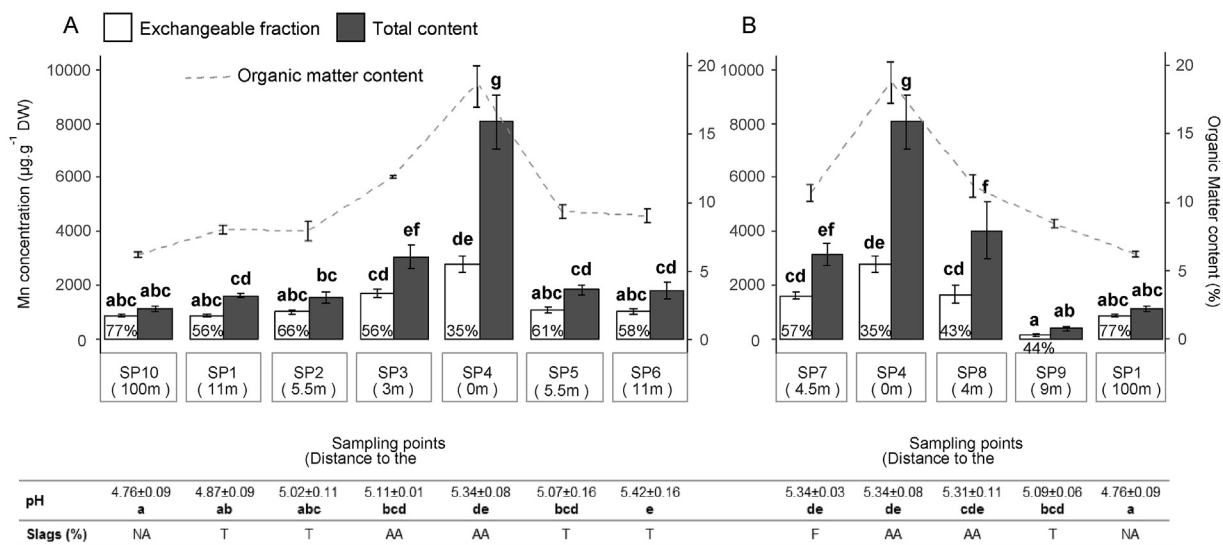


Fig. 4. Total (black bars) and extractable (white bars) manganese concentrations ($\mu\text{g}\cdot\text{g}^{-1}$), the organic matter content expressed in % (dashed line) and the pH value and observed slag content (%) at each sampling point. The NW-SE transect is represented in the left panel (A), and NE-SW is represented in the right panel (B). Different letters correspond to significant differences (p -value<0.05). Code for slag abundance: AA (very abundant, i.e., more than 50%), A (abundant, i.e., between 25 and 50%), F (common, i.e., between 20 and 25%), R (rare, i.e., between 10 and 20%), and T (trace, i.e., less than 10%).

release of Ca^{2+} and hydroxyl (OH^-) ions (Riley and Mayes, 2015; Roadcap et al., 2006). This well-known phenomenon led to the use of slags as a liming agent for soil quality restoration (Lopez et al., 1995; Pinto et al., 1995). Second, we identified serpentine minerals ((Mg, Fe, Ni, Mn, Al, Zn) $[\text{Si}_2\text{O}_5] (\text{OH})_4$) in soils from Antorpe 9 slag heap. Serpentine is a subgroup of minerals belonging to the kaolinite-serpentine group and usually contains divalent cations in octahedrally coordinated sites. This type of mineral is frequently formed after weathering of olivine in rocks. This serpentinization process is also known for magnesian olivines in geological environments (basaltic rock alteration; Muntener, 2010), as well as for zinciferous silicates in surface conditions (Choulet et al., 2016); to our knowledge, the process is rarely demonstrated for ferriferous

and manganese olivines. Finally, along with alkalization and serpentinization, Mn can be considered a release product of slag weathering leading to soil contamination.

From an environmental point of view, this soil contamination raises the question of mobility and potential transfers to abiotic and biotic compartments of the current ecosystem. Chemical extraction using ammonium acetate and hydroxylamine hydrochloride allowed us to estimate the environmental availability of Mn in soils from Antorpe 9. This extractant can extract the exchangeable metal fraction (including water-soluble Mn held on the surface of negatively charged exchange complexes and Mn coprecipitated with the carbonate fraction) and the easily reducible fraction (including oxides) (McAlister and Smith, 1999). This result explains the

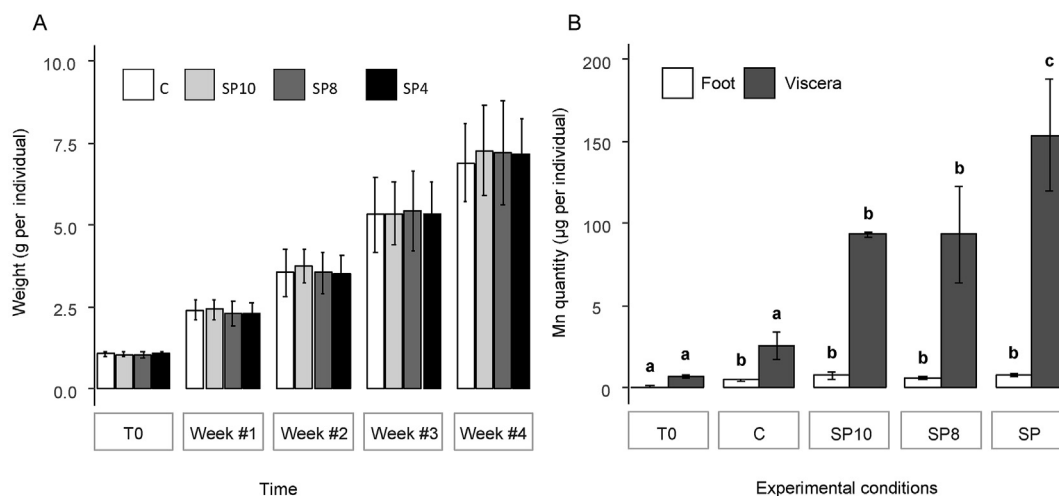


Fig. 5. A: Time course of snail growth (fresh weight) during the 4-week exposure to control conditions (C) and to surface soils from site Antorpe 9. No significant difference (p -value <0.05) between treatments was identified. B: Mn bioaccumulation in *C. aspersus* foot (white) and viscera (black) (expressed in body burdens, $\mu\text{g.snail}^{-1}$) after exposure (for 28 days) to control conditions (C) and to surface soils from site Antorpe 9. T0 corresponds to the metal body burdens in snails at the beginning of the experiment. For each snail part, different letters correspond to significant differences (p -value <0.05).

elevated Mn extraction yields in the reference soil (77%, *i.e.*, $880 \mu\text{g g}^{-1}$). In slag-impacted soils, Mn availability is higher and increases from the edge (approximately $1600 \mu\text{g g}^{-1}$) to the centre of the heap ($2800 \mu\text{g g}^{-1}$). In contrast, the extraction yields decreased, ranging from 66 to 35%. This result may be related to the fractionation of Mn, which relies on (i) the soil characteristics affected by the slag deposit (*i.e.*, alkaline soil pH, high OM and clay content) (Walna et al., 2010) and (ii) the sequestration of a substantial portion of the total Mn concentrations in a soil fraction refractory to the extraction (*i.e.*, the mineral matrix) (Maskall et al., 1995). We have recently confirmed these findings with an in-depth study of the Mn partitioning in soils from Roman and medieval slag heaps (Amnai et al., 2021). We have indeed demonstrated that Mn was mainly found in the reducible (oxides) and residual (slag micro-fragments) fractions of soils. The anomalously low total and extractable concentrations at SP9 may hence be explained by its localization in the vicinity of a small pond, which modifies the hydromorphic conditions of soil (*i.e.*, decreases the redox potential) and subsequently promotes the leaching of Mn (Walna et al., 2010).

4.2. Manganese bioavailability, transfer and toxicity

The terrestrial snail *Cantareus aspersus* is well recognized as a biological indicator (de Vaufléury, 2015), integrating three contamination routes: respiratory (from atmospheric sources), digestive (from plant and soil sources) and dermal (diffusion of metals through the foot epithelium). In addition to the chemical assessment of metal environmental availability, the analysis of metal bioaccumulation in snail tissues constitutes a way to estimate the fraction of the total concentration in soil that is truly transferable in biota, *i.e.*, the metal bioavailability (ISO 17402, 2008). The snail foot is primarily composed of muscles and does not appear, as for other trace elements, to be an important sink for Mn (de Vaufléury et al., 2006). In viscera, the digestive gland (or hepatopancreas) is the main storage organ of Mn, especially in intracellular granules occurring in large numbers in basophil cells (Mason and Simkiss, 1982). The Mn burdens accumulated in snail viscera are in accordance with extractable concentrations and confirm that a substantial portion of the Mn released by slags is effectively bioavailable to soil organisms and particularly snails. Despite the high total and available Mn exposure concentrations in soils, we did

not observe deleterious effects regarding snail survival or growth, as already noticed with artificially contaminated soils (up to $750 \mu\text{g Mn.g}^{-1}$ soil; Bordean et al., 2014). These results are not obvious with respect to ecotoxicity data available in the literature. Indeed, Kuperman et al. (2004) determined laboratory LC_{50} values (concentrations in the soil causing 50% mortality) of $389 \mu\text{g g}^{-1}$, $1970 \mu\text{g g}^{-1}$ and $2575 \mu\text{g g}^{-1}$ for *Enchytraeus crypticus*, *Eisenia fetida* and *Folsomia candida*, respectively. The absence of significant detrimental effects on snails may be related to (i) a misestimation of toxicity metrics estimated using spiked artificial soils (Lock and Janssen, 2001); (ii) the presence in field soils of growth-promoting factors for snails, such as humic substances and oligo-elements (Gomot et al., 1989); and (iii) the ability of *C. aspersus* snails to accumulate and detoxify Mn in their tissues using detoxification processes, such as metal-rich granules (Mason and Simkiss, 1982; Simkiss and Taylor, 1994). However, other sensitive biomarkers at the cellular and subcellular levels (*e.g.*, cytotoxicity; Bradley and Runham, 1996) deserve to be investigated. At the ecosystem scale, snails, as possible prey for numerous invertebrates and vertebrates, may contribute to the diffusion of metals in trophic chains (Moreno-Jiménez et al., 2011).

5. Conclusion

Although slag heaps may be regarded as punctual sources of metal release, their very large number, considerable cumulative volume and spatial coverage make them a significant source of risk (Amnai et al., 2021). The combination of mineralogical, chemical and ecotoxicological approaches allows us to show here that, in addition to the modification of various soil physico-chemical characteristics, such as the pH and OM content, slag weathering over centuries led to the release of metallic contaminants in the surrounding environment. Among them, Mn ranks, based on a combination of its frequency, toxicity, and potential for human exposure, 140th (out of 275) in the substance priority list of the Agency for Toxic Substances and Disease Registry (ATSDR, 2019). We have emphasised not only the environmental availability of this metallic palaeo-pollution but also its bioavailability to soil-dwelling invertebrates, such as snails. Hence, such legacy contaminated sites need to be more clearly identified and included in environmental risk assessment procedures.

CRedit author statement

Quentin Petitjean: Investigation, Analysis and data collection, Statistics, Writing - original draft - review & editing. Flavien Choulet: Data collection and validation, Writing- original draft - review & editing, Funding acquisition, Project administration. Anne-Véronique Walter-Simonnet: Analysis and data collection, Validation, Writing - review & editing. Anne-Lise Mariet: Investigation, Resources. Hervé Laurent: Field campaign, Resources. Patrick Rosenthal: Resources. Annette de Vaufléury: Investigation, Resources. Frédéric Gimbert: Supervision, Conceptualization, Writing- original draft - review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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